REMARKS

Claim 1 has been amended as discussed further below. No new matter is added. Claims 1-6 and 8-9 are pending.

Rejection under 35 U.S.C. § 103(a)

Claims 1-6 and 8-9 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Sode (WO/2002/36779, published May 10, 2002) in view of Herbaud, et al. (Biochim Biophys Acta. 2000, 1481 (1): 18-24) as evidenced by Arslan, et al. (Biochem. Biophys. Res. Commun. 251 (1998) 744-747).

The Examiner first makes the point that the term "enhancing" in claim 1 is not given any weight because there is no explicit definition for "enhanced expression of GDH" in the specification. The Examiner interprets the term to mean that glucose dehydrogenase is expressed (Office Action, page 4, paragraph 1).

The specification teaches *Escherichia* bacterium with improved glucose dehydrogenase (GDH) expression and high GDH activity due to the expression of the cytochrome C maturation (ccm) system in the bacterium. We note that the specification at page 2, last full paragraph recites:

> ...expression of a DNA encoding a glucose dehydrogenase complex of *Burkhorderia cepacia* could be **improved** in an *Escherichia* bacterium by **enhancing** the expression of the ccm system of the bacterium, and thus accomplished the present invention.

In order to more clearly set forth the claimed invention with regards to the improved GDH expression and activity as a result of the coexpression with the ccm system, claim 1 has been amended to "thereby enhancing the expression of a cytochrome c maturation (ccm) system, improving expression of glucose dehydrogenase and providing ~~and~~ high glucose dehydrogenase activity."

Regarding support for "improving expression" and "providing high glucose dehydrogenase activity", the specification teaches that both expression and activity of GDH are improved by coexpression of genes of the ccm operon. For example, at page 2, 2nd to last paragraph, the specification states that "an object of the present invention is to provide a means for **abundantly expressing** an enzyme complex containing the α-subunit and the β-subunit in an

Escherichia bacterium." Page 21 of the specification indicates that this goal has been met. Also, page 20, last full paragraph reports "extremely high GDH activity". In addition, support is found in the present specification at page 2, last full paragraph as reproduced above. Importantly, based upon the description at page 20, one of ordinary skill in the art would understand that "improved" and "high" mean by comparison to a wild strain or a recombinant bacteria which do not "comprise genes of a ccm operon operably linked to a promoter" as claimed (*Burkhorderia cepacia* KS1 and JM109/pTrc99Aγαβ, present specification, page 20).

The amendment to claim 1 is also responsive to the Examiner's comment that "the claims are not directed to "enhanced activity" but to "enhanced expression" (Office Action, page 4, last paragraph) . Claim 1 is now clearly directed to "providing high glucose dehydrogenase activity".

The Examiner continues to argue that the cited art teaches that maturation of cytochrome C was increased in E. coli when expressed with the ccm genes thus increasing the amount of cytochrome C_3 (Office Action, page 4, last paragraph). In response, this was certainly expected as these components are found together in vivo. However, the ccm system is not found together with GDH in vivo. Accordingly the discovery that the ccm system improved expression and provided high activity of GDH was not expected.

The Examiner states that "the prior art indicates that cytochrome C was increased when the α-subunit and the β-subunit of glucose dehydrogenase was co-expressed with the ccm genes" (Office Action, page 5, first paragraph). The basis for this statement is not clear and is, in any case, addressed by the present claim amendment to recite the improved expression and high activity of GDH.

The Examiner asserts that Sode, et al. teach constitutive expression of glucose dehydrogenase in *Escherichia coli* (Office Action, page 6, paragraph 1). However, the presently claimed invention is clearly distinguished from Sode, et al. Sode, et al. do not teach the ccm system and do not teach "improving expression of glucose dehydrogenase and providing high glucose dehydrogenase activity". The disclosure of Sode, et al. corresponds more closely to the control treatments disclosed on page 20 of the present specification (*Burkhorderia cepacia* KS1 and JM109/pTrc99Aγαβ), not the presently claimed invention.

The Examiner states that "the person of ordinary skill in the art would have been motivated to modify the teachings of Sode in with the teachings of Herbaud et al. because "when

the ccm genes are provided on a plasmid together with the structural gene for a mono- and a diheme c-type cytochrome, the cytochrome maturation occurs and seems to be increased" (Herbaud, et al., page 18, col. 2)" (Office Action, page 8, paragraph 3). Accordingly Herbaud, et al. provide motivation to combine the ccm system with a c-type cytochrome but there is no reason to combine the ccm system with a glucose dehydrogenase as claimed by Applicants.

Neither Arslan, et al. nor Herbaud, et al. teach or suggest that expression of a ccm system in E. coli has any effect on a glucose dehydrogenase (or other enzyme) activity. Both Herbaud, et al. and Arslan, et al. merely indicate that, at least in some cases, expression of the ccm genes facilitates production of mature cytochrome c.

In contrast, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). This increase in GDH expression and activity could not have been predicted based upon the disclosure of Herbaud, et al. and Arslan, et al. on stimulation of cytochrome C levels, especially as the stimulation in maturation of cytochrome c was not observed in all cases as discussed previously.

Additionally, Applicants argue that unexpected results were obtained with the claimed combination compared to the prior art. As stated above, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). By co-expressing the enzyme complex including the γ-subunit, the α-subunit and the β-subunit with the ccm genes, the GDH activity in *Escherichia* bacterium increased to a level that was unexpected (present specification, page 20, second full paragraph and pages 20-21, bridging paragraph) compared to expressing the enzyme complex only and the wild type strain. While the activity of the recombinant *E. coli* which also included the genes for the ccm operon (JM109/pTRC99Aγαβ, pBBJMccm) was 32 U/mL, the two controls had activities of only 0.3 (JM109/pTRC99Aγαβ) and 1.4 (*Burkhorderia cepacia* KS1). Such high expression levels could not have been predicted from the cited references.

Herbaud, et al. and Arslan, et al. teach effects on cytochrome C levels, not GDH activity. Furthermore, Herbaud, et al. report that the highest amounts of cytochrome c produced were on the order of 300 μg/L of culture when 0.1 mM δ-aminolevulinic acid was included along with the

ccm system under aerobic conditions (page 22, col. 1, first partial paragraph) which is about the same as what could be produced in *D. vulgaris* (page 22, col. 1, first partial paragraph). Accordingly, Herbaud, et al. do not show levels of cytochrome C production that are unexpected and do not show any effect on activity of an enzyme such as GDH.

Sode, et al. teach the β subunit of GDH but does not suggest a method combining GDH with ccm.

Furthermore, Applicants continue to argue that it was not predictable at the time of the claimed invention that the presence of the ccm operon would result in an increased level of cytochrome C as indicated by Arslan, et al. who teach that inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production of cytochrome c (see page 747, col. 1, last paragraph). They conclude that low levels of ccm gene products must be present already and that addition of ccm genes (pEC86) therefore produced no stimulation.

Although Arslan, et al. teach instances where cytochrome maturation genes (ccm) increased production of both endogenous and foreign c-type cytochromes (see Abstract), in at least one instance stimulation of cytochrome c production by expression of ccm genes was not observed as inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production (see page 747, col. 1, last paragraph).

Accordingly, the claimed microorganism and method provide improvement in both GDH expression and activity as well as enhanced expression of ccm in the presence of the ccm system which could not have been predicted based upon the combination of cited references.

In view of Applicants' amendments and arguments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

Rejection under 35 U.S.C. § 112, first paragraph – new matter

Claims 1-6 and 8-9 are rejected under 35 U.S.C. § 112, first paragraph as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s) had possession of the claimed invention at the time that the application was filed.

The Office Action states that the specification does not specifically mention "enhancing expression of glucose dehydrogenase".

Applicants note that the specification at page 2, last full paragraph recites:

> ...expression of a DNA encoding a glucose dehydrogenase complex of *Burkhorderia cepacia* could be **improved** in an *Escherichia* bacterium by **enhancing** the expression of the ccm system of the bacterium, and thus accomplished the present invention.

Moreover, a standard dictionary definition of "enhance" (attached) which indicates that "enhance" means "to ...improve in value, quality, desirability, or attractiveness". Certainly the words are used interchangeably, at least in some instances.

Claim 1 has been amended as : "An *Escherichia* bacterium, comprising DNAs encoding the α-subunit and the β-subunit of glucose dehydrogenase of *Burkhorderia cepacia* in an expressible form and further comprising genes of a ccm operon operably linked to a promoter, thereby enhancing the expression of a cytochrome c maturation (ccm) system, improving expression of glucose dehydrogenase and providing ~~and~~ high glucose dehydrogenase activity."

According to the specification, both expression and activity of GDH are enhanced by coexpression of genes of the ccm operon. For example, at page 2, 2nd to last paragraph, the specification states that "an object of the present invention is to provide a means for **abundantly expressing** an enzyme complex containing the α-subunit and the β-subunit in an *Escherichia* bacterium." Page 21 of the specification indicates that this goal has been met. Also, page 20, last full paragraph reports "extremely high GDH activity". Based upon the description at page 20, one of ordinary skill in the art would understand that "improved" and "high" mean by comparison to a wild strain or a recombinant bacteria which do not "comprise genes of a ccm operon operably linked to a promoter" as claimed (*Burkhorderia cepacia* KS1 and JM109/pTrc99Aγαβ).(present specification, page 20).

In view of Applicants' amendments and arguments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-6 and 8-9 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that the phrase "enhancing the expression of...glucose dehydrogenase" in claim 1 renders the claim indefinite.

Claim 1 has been amended as : "An *Escherichia* bacterium, comprising DNAs encoding the α-subunit and the β-subunit of glucose dehydrogenase of *Burkhorderia cepacia* in an expressible form and further comprising genes of a ccm operon operably linked to a promoter, thereby enhancing the expression of a cytochrome c maturation (ccm) system, improving expression of glucose dehydrogenase and providing ~~and~~ high glucose dehydrogenase activity."

According to the specification, both expression and activity of GDH are enhanced by coexpression of genes of the ccm operon. For example, at page 2, 2nd to last paragraph, the specification states that "an object of the present invention is to provide a means for **abundantly expressing** an enzyme complex containing the α-subunit and the β-subunit in an *Escherichia* bacterium." Page 21 of the specification indicates that this goal has been met. Also, page 20, last full paragraph reports "extremely high GDH activity". Based upon the description at page 20, one of ordinary skill in the art would understand that "improved" and "high" mean by comparison to a wild strain or a recombinant bacteria which do not "comprise genes of a ccm operon operably linked to a promoter" as claimed (*Burkhorderia cepacia* KS1 and JM109/pTrc99Aγαβ).(present specification, page 20).

In view of Applicants' amendments and arguments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not

reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

In view of Applicants' amendments to the claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: Oct. 10, 2008

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ATTACHMENT

va=enhance

One entry found.

enhance

Main Entry: **en·hance**
Pronunciation: \in-'han(t)s, en-\
Function: *transitive verb*
Inflected Form(s): **en·hanced**; **en·hanc·ing**
Etymology: Middle English *enhauncen,* from Anglo-French *enhaucer, enhauncer,* from Vulgar Latin **inaltiare,* from Latin *in* + *altus* high — more at OLD
Date: 13th century

1 *obsolete* **:** RAISE
2 : HEIGHTEN, INCREASE; *especially* **:** to increase or improve in value, quality, desirability, or attractiveness <*enhanced* the room with crown molding>
— en·hance·ment \-'han(t)-smənt\ *noun*

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